

PHYSIOLOGICAL POLARITY AND DOMINANCE IN THE HOLDFAST SYSTEM OF *CORYMORPHA*.

C. M. CHILD.

HULL ZOÖLOGICAL LABORATORY, THE UNIVERSITY OF CHICAGO.

Corymorpha palma is found on tidal flats and anchors itself on the substratum of soft mud or fine sand by means of a large number of filamentous, stolon-like structures covered with a delicate perisarc, which extend from the basal region of the hydroid and serve as holdfasts. Torrey ('04, '07, '10a, '10b) has described the structure, the embryonic and reconstitucional development and some of the reactions of these holdfasts, viz., their extremely rapid elongation, the ameboid activity of their tips, their positive geotropic reaction and the ability of actively developing holdfasts to inhibit the development of others distal to them.

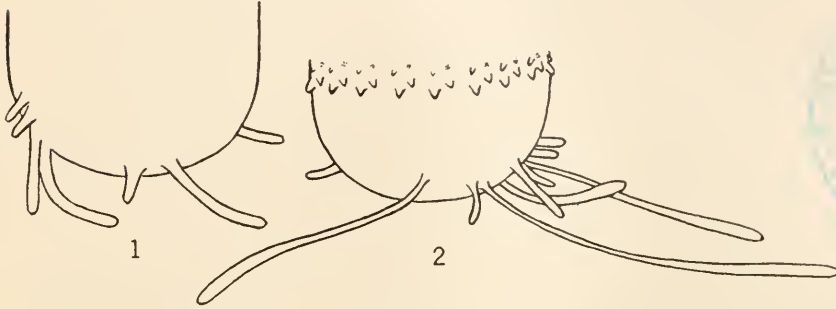
The holdfasts are much more slender than the usual hydroid stolon, being only about 0.1 mm. in diameter at the tip which is the region of greatest diameter except in early stages, but they are similar to other hydroid stolons in appearance and development. Apparently, however, they are somewhat more specialized than the ordinary stolon for, so far as known, they do not transform into, or give rise to hydranth-stem axes when isolated from the parent stem, but always remain holdfasts. If they really lack the capacity to give rise to hydranth-stem axes they should, strictly speaking, be designated as holdfasts rather than as stolons. Nevertheless it seems evident that they represent merely a specialized form of stoloniac axis. Each holdfast evidently represents a physiological axis and possesses a definite polarity which appears in its form, the manner of its development and its final exhaustion. Moreover, the holdfast system of the individual hydroid shows certain definite relations to the polarity of the whole, both in its origin and in the relations of its members. The present paper is primarily concerned with those aspects of holdfast life which are related to physiological polarity, viz., the manifestations of polarity in the individual holdfast and the relations of the holdfast system to the polarity of the whole animal.

THE INDIVIDUAL HOLDFAST AS A PHYSIOLOGICAL AXIS.

The Gradient of the Holdfast.—The holdfast originates as a bud in the basal region of the hydroid and develops as an axis with a growing tip. Like other buds, it arises as a localized region of cellular activity which decreases in intensity from a central region peripherally. As the holdfast develops the most active region necessarily becomes the tip and the radial gradient in activity which constitutes the first step in the development becomes an axial gradient with its high end at the tip. The existence of this gradient from the earliest localization of the holdfast bud has been demonstrated by its differential susceptibility to various agents, by differential vital staining, by differential reduction of vital dyes and KMnO_4 and by the differential in the indophenol reaction. All the methods used agree in showing that the tip of the holdfast is the high end of the gradient. The results obtained on the animal as a whole with these various methods have been described in an earlier paper (Child, '26a) and since all methods agree as regards the holdfast, it seems unnecessary to give the data in detail. Moreover, the gradient is obvious in the general behavior of the holdfast. It may be noted, however, that the various agents penetrate the very thin perisarc almost at once and with those agents which afford a means of following the penetration directly by staining it is found that the older perisarc farther from the holdfast tip is usually penetrated before that at the tip. Evidently the very conspicuous gradient of the holdfast cannot be ascribed to differences in permeability of the perisarc.

The holdfast bud or primordium may persist for a long time without appreciable growth beneath the perisarc of the stem, but remains capable of very rapid outgrowth when conditions permit. As a bud it usually tapers slightly toward the tip, but when outgrowth begins its diameter is for a time about the same throughout its length. With further outgrowth and particularly after it comes into contact with the substratum, the tip retains its diameter, or the diameter may appear to increase somewhat because of flattening of the tip on the substratum, but proximal to the tip the diameter gradually decreases until the proximal portions of the longer holdfasts are extremely slender threads. This decrease in diameter is the result of gradual atrophy of the cells in the more

proximal regions of the elongating holdfast until there is nothing left except the tube of very delicate perisarc secreted by the holdfast as it elongates and within it traces of cellular debris. Figs. 1 and 2 show various stages in the development of holdfasts. In these and all following figures perisarc is indicated by a heavy line.



FIGS. 1 AND 2. Activation and outgrowth of holdfasts. Fig. 1, proximal cut end of stem piece 1 1/2 hours after section, showing activation and outgrowth of holdfasts from very early bud stages present before section. Fig. 2, a proximal cut end 24 hours after section, showing a stage of development of a second set of holdfasts 3 hours after removal of first set. A new zone of holdfast buds has begun to develop a short distance distal to the active holdfasts.

Atrophy of the more proximal levels is characteristic of the true stolons of other hydroids (Child, '23) as well as of these holdfasts, another fact which indicates the physiological similarity of true stolon and holdfast. It was pointed out in the paper just referred to that this atrophy is clearly a consequence of the gradient in the stolon. While the outgrowth is short all parts obtain nutrition from the hydroid body, but it appears that after a certain length is attained this is no longer possible and from this stage on the outgrowth is gradually undergoing starvation and reduction. Very generally, if not always, in the starvation of the simpler animals the parts which are most continuously and most intensely active decrease in size less rapidly than the less active because they are able to live and more nearly maintain themselves at the expense of the less active. Microscopic examination of the cells of the longer holdfasts shows that those at or near the tip appear to be in good condition while evidences of inanition increase with increasing distance from the tip. Toward the proximal end the

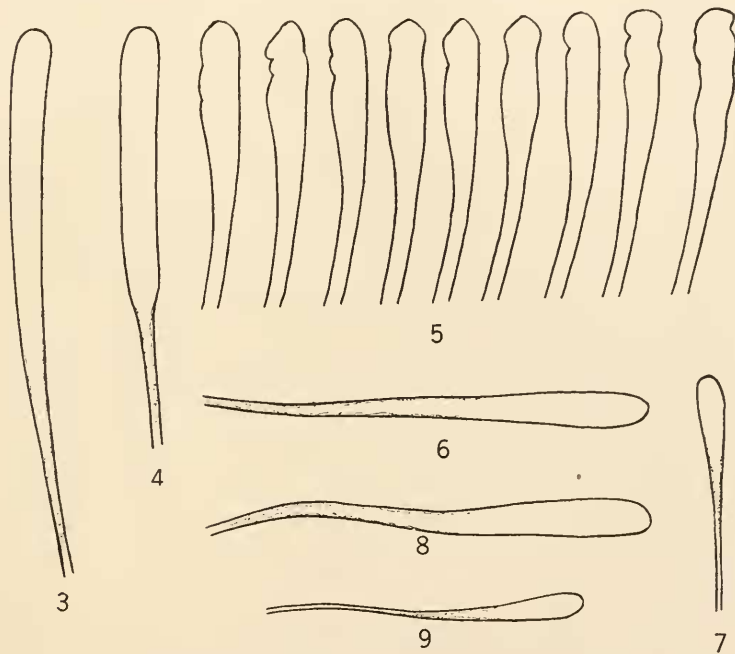
cells become more transparent and the holdfast has decreased in diameter more than the perisarc so that only strands of protoplasm here and there are in contact with the perisarc. Finally at a greater or less distance from the tip nothing remains within the perisarc but a few granules. The transition from the region of good condition at the tip to that of complete atrophy is usually very gradual, as indicated in Fig. 3, but occasionally it is rather abrupt, particularly in the earlier stages of the atrophy (Fig. 4). The methods used to demonstrate the gradient indicate that such differences are due to individual differences in length and slope of the gradient in the holdfast and such differences are undoubtedly associated with differences in physiological condition and rate of elongation in the different holdfasts.

The holdfast, like the stolon of other hydroids is obviously a gradient in which the characteristic process, at least in the earlier stages, is continued growth of the tip. As a growth gradient it is also similar to rhizoids, roots and various forms of hairs in plants and differs from the chief plant axis only in its inability to give rise to buds.

• *The Motor Activity of the Holdfast.*—Holdfasts growing rapidly from the cut end of a stem as in Figs. 1 and 2 often show slight motor activity before they come into contact with the substratum. Their movement consists in bending in various directions and in some cases slight contraction and extension are observed. All such movements are slow, much slower than tentacle movements. Motor response of holdfasts to direct external stimulation has not been tested.

As soon as the holdfast comes into contact with the substratum it adheres and further motor activity is limited to the tip. In nature the holdfasts burrow through the mud, but in glass containers in the laboratory elongation after contact takes place along the surface of the substratum. (Torrey, '10b) has noted that the elongating holdfast is positively geotropic. After attachment, however, the holdfast tip often shows more or less amœboid activity, as Torrey ('04, p. 416) has also noted. Elongated pseudopodia have not been observed, but the distal region may undergo change in shape, the activity decreasing from the tip proximally. Fig. 5 shows a series of outline drawings of a holdfast tip at in-

tervals of one to two minutes and serves to indicate the character and range of amœboid activity observed. In general this activity has seemed to be greater in holdfast tips farther away from the stem, rather than in the earlier stages of outgrowth, but it cannot be positively asserted that this is the general rule. Occasionally the extreme tip has been seen to undergo slight retraction, 0.03–0.07 mm., followed after a minute or two by renewed advance. So far as observed, the amœboid activity occurs chiefly at the extreme tip and decreases rapidly in the proximal direction and at a distance of 0.3–0.5 mm. from the tip is no longer visible.



FIGS. 3–9. Individual holdfast tips. The shrunken appearance of the proximal cœnosarcal regions is indicated by dotted lines inside the heavy lines indicating perisarc. Fig. 3, a holdfast which has covered a distance of 7–8 mm., showing gradual decrease in diameter and increase in degree of atrophy in proximal direction. Fig. 4, a holdfast which has progressed 7–8 mm., showing rather abrupt decrease in diameter and increase in degree of atrophy. Fig. 5, a series of outlines of a holdfast tip sketched at intervals of 1–2 minutes to show the amœboid change in form. Fig. 6, a holdfast which has covered 15 mm. Fig. 7, a holdfast which has covered 20 mm. and is approaching exhaustion. Fig. 8, an earlier, and Fig. 9 a later stage of a completely isolated holdfast.

The Later Stages of Holdfast Development.—When the holdfast attains a certain length which varies in different holdfasts, the atrophy of the more proximal regions brings about complete separation of the distal portion of the cœnosarc from the hydroid stem. The beginning of this atrophy usually becomes evident in the decrease in diameter of the proximal region when the holdfast has attained a length of 2–3 mm. (Fig. 2) and by the time it has reached a length of 4–8 mm. the living cœnosarc of its distal end is apparently completely separated from the parent stem, the perisarc tube being the only connection (see Figs. 12, 13).

Advance of the tip does not cease when this isolation occurs. The more proximal portions evidently serve as nutrition for the distal parts and the progress of the tip with the secretion of perisarc continues with gradual decrease in length and diameter of the cœnosarc until finally exhaustion occurs. Figs. 3, 4, 6, 7 illustrate the decrease in size of the tip. Figs. 3 and 4 represent the distal portions of holdfasts which have grown a distance of 7–8 mm. from the stem, Fig. 6 is a distal end 14–15 mm. and Fig. 7 another 20 mm. from the stem and almost exhausted. Advance does not continue much beyond the stage of Fig. 7. Apparently the gradient differences at this stage are no longer great enough in the small fraction of the original gradient which remains so that the tip can maintain itself and continue to advance at the expense of lower levels, *i.e.*, the degree of starvation has become almost as great at the tip as elsewhere. After its advance ceases the tip may continue to live and decrease in size still further for at least a week, perhaps more.

That the continued advance of the tip is entirely independent of connection with the parent hydroid is evident from the fact that it is not at all affected by physical isolation of the tip from the hydroid. Fig. 8 represents a holdfast from which the perisarc tube connecting it with the parent stem has been cut away. This tip continued to advance, secreting the perisarc tube exactly as if connected with the stem. Fig. 9 shows a later stage, approaching exhaustion. When a stem is removed after giving rise to holdfasts the latter remain attached to the substratum and continue to advance until they are exhausted.

In this continued advance of the tip after complete isolation

from the hydroid the holdfast also resembles the true stolon of other hydroids (Child, '23). In both the advance continues long after physiological connection with the hydroid stem is lost and until the *cœnosarc* is reduced to a small fraction of its original size. Even when the advance ceases the cells of the tip are visibly in better condition than those of lower levels.

In the laboratory holdfasts have been observed to advance over a distance of 2-3 cm. before exhaustion and the binding of the mud about the base of the hydroid in nature indicates that under natural conditions they may cover a somewhat greater distance than this.

Torrey ('04, p. 416, '07, pp. 277-8) regards advance of the holdfast tip as due primarily to amoeboid activity at the tip which stretches the holdfast. He states, however, that when stems are suspended free in water the holdfasts extend in all directions. On cut stems also the holdfasts elongate very rapidly before they come into contact with the substratum at all (Figs. 1 and 2). Such elongation when the tip is not in contact certainly cannot be due to amoeboid activity for the tip cannot exert tension. Moreover, the volume of the holdfast *cœnosarc* increases very rapidly and very greatly during the earlier stages. And finally, the progress of the tip is not necessarily in a straight line, but the direction may undergo frequent change, even on smooth glass so that the perisarcal tube is sinuous or forms a circle or a spiral (see Figs. 12 and 13). If any actual stretching occurs it must take place only very near the tip and must very soon reach a limit for the perisarc is not torn away from the substratum and straightened and the *cœnosarc* is not left behind. It seems certain that at least the earlier stages of holdfast elongation are not due merely to the amoeboid activity of the tip. To all appearances extensive growth occurs, although it is conceivable that cells migrate from the parent stem into the holdfast. Evidence of such migration, however, is lacking.

To what extent the later advance of the holdfast tip after isolation of its *cœnosarc* from that of the parent stem may be due to amoeboid activity and to what extent to actual growth of the tip at the expense of more proximal levels is less readily determined. The appearance of the cells at the tip suggests continued growth,

but the very rapid advance suggests amœboid activity. The perisarc is certainly continuously secreted as the tip advances, rather than elongated by stretching. The planula of *Corymorpha* often progresses for some distance over the substratum leaving a delicate perisarc tube behind it as it travels (Child, '26a). In that case the advance is evidently due to motor activity rather than growth. It seems improbable, however, that the holdfast changes completely its activity from growth to amœboid activity after it comes into contact with the substratum. Amœboid activity has been observed in tips which are approaching exhaustion and have almost ceased to advance. In the light of all the facts it seems probable that more or less growth of the tip at the expense of proximal levels does occur even after isolation from the stem, although amœboid activity may play a part in the advance of the tip in later stages.

The continued advance of the tip until exhaustion occurs and its maintenance in good condition at the expense of more proximal regions with progressive atrophy of the proximal parts and the amœboid activity of the tip are evidently all expressions of the physiological gradient characteristic of the holdfast. In fact, it does not seem possible to account for its behavior except in terms of a gradient. Moreover, the gradient accounts adequately and entirely for all the phenomena of polarity which the holdfast exhibits.

The Rate of Advance of the Holdfast.—The advance of the holdfast is so rapid that with a low magnification and an ocular micrometer the advance from minute to minute can be directly observed. Different holdfasts show different rates of advance, but in general the rate decreases very greatly in the later stages and advance finally ceases. The following table gives a few characteristic measurements of the rate of advance. Nos. I., II. and III. are the three longest holdfasts of Fig. 2 measured at a stage a few minutes later than that represented in the figure, *i.e.*, about three hours after section of the stem and activation of the holdfasts. These early stages were elongating at the rate of a millimeter in seventeen to twenty-two and one half minutes, the highest rates observed. Nos. IV., V. and VI. are holdfasts twenty-four hours old, growing from an intact base after removal of the original holdfasts. In these the rates of advance are much slower

TABLE I.
RATES OF ADVANCE OF HOLDFAST TIPS.

| | | Distance of Tip from Parent Stem in Mm. | Time in Minutes and Seconds to Cover 10 Micrometer Divisions (0.33 mm.) |
|---|-----|--|---|
| Holdfasts from cut end of stem 3 hrs. after section..... | I | 3 | 7:30 |
| | II | 4 | 6:40 |
| | III | 5 | 5:40 |
| Holdfasts 24 hrs. old from intact basal end after removal of original holdfasts..... | IV | 8 | 14 |
| | V | 15 | 20 |
| | VI | 20 | 46 |

and it will be observed that they decrease to a marked extent with increasing length of the holdfast. These three cases are the three holdfasts of Figs. 4, 6 and 7 at the stages figured. Fig. 7, No. VI. in the table, was approaching exhaustion at the time of measurement and its rate of advance is only about one eighth that of No. I. A number of other measurements made all fall within the extremes of the table. Torrey ('04) mentions a rate of nine micra per minute in one case; this would equal 0.33 mm. in more than thirty-three minutes, a rate much slower than those of the earlier stages in the table. Elsewhere (Torrey, '07) he mentions a rate of 15 mm. per day which is almost midway between the rates of Nos. V. and VI. of the table. The rates in Table I. are all from holdfasts in contact with the substratum. The rates of the earlier stages seem high for a growth process, but rates approaching these are found in holdfasts which are not yet in contact with the substratum. Fig. 1, for example, shows a basal cut end 1 1/2 hours after the cut was made. Before the section only very early holdfast primordia were present but within the short time following section some holdfasts attained a length of 1 mm. before their tips came into contact with the substratum. Most of the length of 1 mm. was covered during the last half hour of the period. Except for possible migration of cells from the stem into the holdfast this elongation appears to be due to growth and

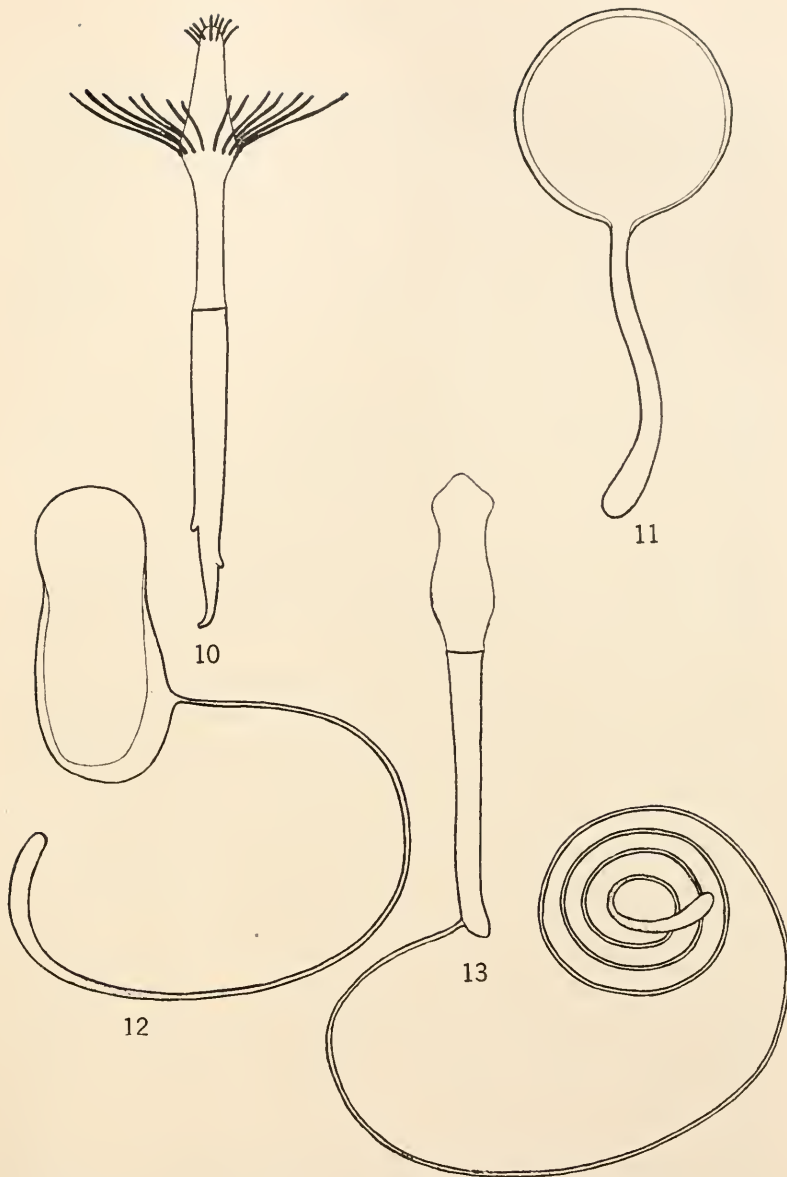
the rate of elongation is nearly as high as in some of the holdfasts after contact. The holdfast appears to be primarily a growth gradient with amoeboid activity at its tip.

DOMINANCE AND PHYSIOLOGICAL ISOLATION IN THE ORIGIN AND DEVELOPMENT OF THE HOLDFAST SYSTEM.

In later stages of the hydroid development the holdfast buds arise in regular order along the entodermal canals in the basal region and show in their origin and development certain definite relations to the polarity of the whole animal and to each other.

Early Normal Development.—The first holdfasts arise as minute buds at or near the basal end of the perisarcal region of the young hydroid, but they appear only after the young hydroid has become attached by its perisarc and they remain as minute buds under the perisarc for some time after their appearance. Under good conditions the first holdfasts appear only when the hydroid has attained the stage shown in Fig. 10 and often not until still later. In general holdfast development begins at or near the basal end and progresses distally from it, but the early holdfasts are more or less irregularly scattered in position. In vigorous, actively growing young animals the holdfast buds do not grow out through the perisarc of the stem at once after their formation, but develop very slowly for a time, remaining buds and not beginning active outgrowth until the animal attains a considerable length. During this earlier stage they are merely primordia of holdfasts and play no part in attaching the animal unless they happen to be terminal. This very slow development until the animal attains a certain length suggests that the holdfast buds, like the stolons of other hydroids are inhibited in development by apical regions until the increase in length of body brings about a certain degree of physiological isolation.

Premature Development of Holdfasts under Inhibiting Conditions.—Under conditions which inhibit development to a slight extent holdfasts may not only appear as buds, but may actually grow out and attach themselves at much earlier stages of development than those at which they normally appear. In low concentrations of KCN, alcohol, ether, etc. and even when kept in sea water several centimeters deep without change or aeration the



FIGS. 10-13. Development of holdfasts in young animals. Fig. 10, young hydroid in stage at which holdfast buds usually first appear under normal conditions. Fig. 11, outgrowth of a holdfast from a blastula under slightly inhibiting conditions. Fig. 12, a planula with holdfast which appeared in blastula stage under inhibiting conditions. Fig. 13, slightly inhibited young hydroid with long holdfast which appeared in the early planula stage. Hydroid and holdfast are figured in the same plane, though in nature the plane of holdfast elongation is at right angles to the hydroid axis.

holdfasts develop in the planula or even in the blastula stage. Fig. 11 shows a blastula with holdfast. Fig. 12 a planula with holdfast which began its development in the blastula stage, Fig. 13 an early hydroid with holdfast which appeared in the early planula stage twenty-four hours before the stage of the figure. The spiral growth seen in Fig. 13 is of frequent occurrence in the holdfasts, but the conditions which determine it are unknown. These three, as well as numerous other similar cases observed are from material developing at the bottom of 5 cm. depth of standing water without aeration or change since early cleavage stages. As compared with development in frequently changed, well aerated water, this material was distinctly inhibited in development. Many similar cases of premature development of holdfasts have been observed with low concentrations of various inhibiting agents. In fact, the early formation and outgrowth of holdfasts is the characteristic effect of a slight degree of inhibition or retardation of development.

In this connection it may be recalled that development of stolons instead of the hydranth-stem axis from the planula has been experimentally determined in a campanularian hydroid, *Phialidium gregarium*, by various inhibiting conditions (Child, '25). In that form both ends of the planula may give rise to stolons. In *Corymorpha* the premature development of holdfasts has been observed only from the basal region and the hydranth-stem axis has developed sooner or later, but always retarded. It has been pointed out elsewhere that the polarity of the tubularian hydroids is apparently less readily altered than that of the campanularian (Child, '19, '25, '26a) and the persistence of the planula polarity in *Corymorpha* under inhibiting conditions constitutes further evidence along the same line.

The much earlier appearance and outgrowth of the holdfasts under slightly inhibiting than under normal conditions shows that the inhibiting conditions favor in some way their development. But it is evident that the inhibiting conditions are not in themselves necessary for development of holdfasts, since in larger older animals the holdfasts develop very rapidly in the best possible laboratory environment (Figs. 1, 2). As regards the manner in which the inhibiting conditions induce premature development

of holdfasts, two possible factors suggest themselves. First, the inhibiting conditions may directly affect the lower end of the primary gradient which is to become the basal region and lower its metabolic or physiological level so that conditions favorable to holdfast development arise earlier than they would under normal conditions, in which they appear only after the animal has become considerably older and its metabolism has undergone decrease. It has been shown elsewhere (Child, '27*a*, *b*) that basal regions may be determined and localized by local inhibiting conditions and it is possible that the direct effect of the inhibiting conditions in decreasing the activity of the lower end of the gradient may be concerned in determining holdfasts at an earlier stage of development than that at which they normally appear.

A second possible factor in the premature development of holdfasts under inhibiting conditions is an increase in the degree of physiological isolation of the lower end of the gradient from apical dominance in consequence of the effect of the inhibiting conditions on the apical region. The fact that the holdfasts are not only prematurely determined as buds but also develop prematurely with great rapidity indicates that the effect of the inhibiting conditions on their development is, at least in large part, indirect rather than direct, *i.e.*, an increase in the degree of physiological isolation of the basal region from apical dominance resulting from the inhibiting action of the conditions on the apical region. Under normal conditions such physiological isolation does not occur until a much later developmental stage when the length of the hydroid begins to exceed the range of that degree of dominance necessary for complete inhibition of holdfast development, and even then their development is very slow until a considerably greater length of stem is attained, which apparently permits a greater degree of physiological isolation. The dominant apical region is more susceptible than the less active basal region to the inhibiting conditions (Child, '26*a*) and its dominance over the basal region is decreased, consequently holdfasts are able not only to appear as buds, but to grow out at once. The holdfast evidently represents a new physiological axis which can be formed in the basal region of the hydroid only when a certain degree of physiological isolation from the dominance of the apical region

occurs. A similar relation between hydranth region and stolon exists in *Tubularia* (Child, '15, pp. 91, 92, Figs. 42, 43) as well as in other hydroids, but in those forms the stolon tip transforms into a hydranth-stem axis as soon as the degree of isolation is sufficient.

This interpretation of holdfast development does not conflict in any way with the fact that new basal ends on which holdfast buds may develop and grow out are often determined in pieces undergoing reconstitution by the inhibiting conditions associated with contact or nearness to the bottom (Child, '26*b*), especially with the addition of inhibiting agents (Child, '27*a*). In normal development and in many cases of reconstitution the basal region is apparently largely or wholly determined by internal factors as a secondary gradient at the low end of the primary gradient (Child, '26*b*). Apparently it represents a region sufficiently isolated physiologically from apical dominance to develop a new gradient opposite in direction to the old and to grow in length, but still to some extent under apical dominance since it possesses the capacity to develop a hydranth-stem axis from its proximal end (Child, '26*b*, p. 786), but does not do so except after separation from the apical and inhibition of its own distal region. The late appearance and slow development of holdfasts in normal young animals suggests that a further increase in the degree of physiological isolation of the basal region is necessary for holdfast formation. Since a condition of relatively low metabolism is evidently the essential factor in determining a basal region, local external inhibiting conditions may determine such a region or assist in determining it, by establishing the proper metabolic conditions. Whether or when holdfasts shall develop on a basal region thus determined apparently depends on the degree of physiological isolation from the dominant apical region which is attained in such a region. In the piece undergoing reconstitution the new apical region is often not sufficiently developed at the time the basal region is determined to prevent the appearance of holdfast buds; but apparently it often does inhibit their further outgrowth for usually they develop very slowly until the animal attains a certain length and in the very small individuals from short pieces they may remain buds indefinitely. In both reconstitution and embryonic development, however, inhibition of hydranth development

favors their early outgrowth unless the inhibiting conditions are sufficient to inhibit the holdfast buds directly.

Physiological Dominance and Isolation among the Holdfasts.—As noted above, the earlier holdfasts are scattered about the basal end, but after the longitudinal entodermal canals develop, the later holdfast buds arise in regular order along these canals, usually two rows to a canal, forming a definite zone surrounding the stem several millimeters from the basal tip. In this zone the first buds to appear and the most advanced in development at any given time are those nearest the basal tip and bud development progresses distally. Fig. 14 shows the buds on a single canal as they appear in the older animals and in Fig. 2 a new zone of buds is developing distal to a cut end.



FIG. 14. The system of holdfast buds of a single entodermal canal as it appears in older animals.

This definite order of development and spatial arrangement also suggests the existence of a physiological relation between the individual holdfasts of the system and Torrey has observed that the removal of the older holdfasts accelerates the development of those near the wound on the distal side (Torrey, '10a, p. 217). In the normal development of the animal the holdfasts nearest the basal tip grow out first when the animal attains a certain length. After protoplasmic connection between their tips and the stem is severed by atrophy of the connecting region resulting from elongation the buds next in order distally grow out and so on. Meanwhile new buds may begin to develop at the distal end of the bud zone. This zone therefore gradually changes its position in the

distal direction and in the larger individuals is often entirely above the surface of the mud in which the basal end is buried. All these facts indicate the existence of a relation of dominance and subordination in the holdfast system, the holdfasts at the proximal end dominating those distal to them. In this relation the holdfasts which develop from the higher levels of the basal gradient dominate those of lower levels, just as in other similar gradients, *e.g.*, many plant axes.

After the zone of holdfast buds has developed, the buds of any level of this zone can be activated at once by section of the stem just proximal to them. Such section removes the proximal dominant buds or holdfasts and the most proximal buds remaining grow out and become dominant. This activation and outgrowth take place very rapidly. Fig. 1, for example, shows the proximal end of a stem 1 1/2 hours after section through the distal region of the holdfast zone. Before section the holdfast buds in this region were in very early stages like those of the upper third of Fig. 14. Within the short time of 1 1/2 hours some of these buds attained a length of almost 1 mm.

Fig. 2 shows a stem in which a second set of holdfasts is developing after removal of the first set which developed after removal of the basal end 24 hours preceding the stage figured. The first set of holdfasts was allowed to develop for 21 hours at which time those which developed first had lost coenosarcial connection with the stem and others distal to them were developing. Then all developed holdfasts were removed. Fig. 2 shows the development attained by the new set three hours after removal of the old. Fig. 2 also shows a new zone of holdfast buds developing at a short distance from the proximal end. This zone has developed within 24 hours since it was not present at the time of section. Such a new bud zone has been observed in other similar cases and in all, as indicated in Fig. 2, it arises a short distance distal to the actively growing holdfasts, not immediately adjoining them. This position is characteristic and again indicates that these rapidly growing holdfasts dominate a certain length of stem in such a manner as to prevent development even of other holdfast buds within that region. The following experimental data

show that the rapid reaction following section of stem or removal of holdfasts in the cases above described is not exceptional.

I. Twenty stem pieces comprising the proximal fifth of the naked region and the distal half of the perisarcal region were cut from newly collected, relatively young individuals 25–30 mm. in length. These pieces were approximately one fourth the total stem length. They possess only very early holdfast buds or no visible buds. After 1 1/2 hours three pieces were attached by their holdfasts, some of which were 1 mm. long (Fig. 1) and eight others showed outgrowing holdfasts at the proximal end. After 14 hours nineteen pieces were attached; of these fifteen showed a maze of holdfasts about their bases, some of them several mm. in length, and four pieces were attached by the perisarc without holdfast outgrowth.

II. Six pieces from the preceding experiment were detached and all developed holdfasts were removed from the proximal end after 21 hours growth. One hour later two cases showed outgrowing holdfasts, one a single holdfast 0.23 mm., the other, two, each 0.17 mm., both attached to the bottom. After 1 1/2 hours four pieces showed outgrowing holdfasts and three were attached by them. After 2 hours all six showed growing holdfasts and four were attached. One holdfast was 1.3 mm. in length. After 3 hours four were attached. Fig. 2 represents one of the pieces at this stage. After 4 hours, five, and after 9 hours, all were attached by holdfasts. Five of the six pieces developed a second zone of holdfasts buds like Fig. 2 within 33 hours or less.

III. From ten newly collected individuals 50–60 mm. in length hydranths and basal ends, including all visible holdfast buds, were removed. The earlier stages of new holdfast development were not observed, but after 18 hours eight were attached with numerous holdfasts some 3–4 mm. in length. Two pieces were still unattached, but show early developing holdfast buds. In this experiment preformed buds were absent but the formation and development of new holdfasts to a length of 3–4 mm. has occurred within 18 hours.

IV. Ten stems similar to III. After 22 hours five were attached and some holdfasts were 3–4 mm. long. All five showed a new zone of holdfasts developing 1–2 mm. distal to the outgrowing

holdfasts. Five were unattached, one with early holdfasts, four without holdfasts. After 46 hours all had developed holdfasts and become attached.

V. In this experiment four larger animals, 60–70 mm. in length, which had been 6 days in the laboratory, were used. In these the holdfast bud zone had already developed above the level of the mud, (see p. 30). These were the largest individuals obtainable during the summer of 1926, when most of the observations on holdfasts were made. The basal ends were removed at such level as to leave 6–8 rows of preformed holdfast buds distal to the cut end, and the hydranths were also removed. After 2 hours the most proximal one or two rows of buds were elongating and after 8 hours all four pieces showed holdfasts 0.5–0.8 mm. in length and two were attached by their holdfasts. The much slower outgrowth in this, as compared with other experiments is doubtless due to the six day period in the laboratory without food preceding the experiment.

These few experiments are sufficient to indicate the extremely rapid activation of preformed holdfast buds and development of new buds and holdfasts after removal of levels proximal to them.

CONCLUSION.

The *Corymorpha* holdfast is a tertiary axis which develops from the secondary axis which constitutes the basal region. It resembles the stolons of other hydroids in appearance and in its growth and development, but is apparently more specialized than the true stolon, since it is not known to give rise to a new hydranth-stem axis as the stolon does when isolated. All experimental methods applied to the holdfast indicate that it represents a physiological gradient with high end at the tip, like a hydroid stolon, a plant root or rhizoid and many other physiological axes. Moreover, the developmental behavior of the holdfast, the greater amoeboid activity of its tip as compared with other parts, the maintenance in good condition of distal, at the expense of proximal regions, even during gradual reduction by starvation, all constitute further evidence that it is a gradient. And there is absolutely nothing to indicate, and no ground for assuming that its axiate character has any other basis than this gradient.

The secondary gradient which characterizes the basal region apparently represents a certain degree of physiological isolation from apical dominance and the slow appearance and development of the holdfasts under normal conditions, as compared with their premature development under inhibiting conditions suggest that a still higher degree of physiological isolation is necessary for their development than for the determination of the basal end. Since the apical region is more susceptible to inhibiting conditions than the basal (Child, '26a), these conditions decrease apical dominance without greatly affecting the holdfasts themselves, *i.e.*, they inhibit the internal factors which inhibit or retard the outgrowth of the holdfasts and so increase the degree of physiological isolation in the holdfast region.

When active development of the holdfasts does begin, a relation of dominance and subordination arises within the holdfast system. The most proximal holdfasts, *i.e.*, those nearest the basal tip, are the first to appear, as might be expected, since the degree of physiological isolation must be greater there than in more distal regions. These first holdfasts dominate buds distal to them and prevent or retard their development. Removal or outgrowth and separation of these most proximal holdfasts permits the further development of those next distally and so on. This relation is essentially identical with that found in many axiate complexes of plants.

Concerning the nature of this dominance in *Corymorpha* nothing definite can be said. The very wide occurrence in both plants and animals of essentially identical phenomena of dominance, subordination and physiological isolation and the apparently primary dependence of dominance on high metabolic activity, rather than on any particular kind of activity indicate the non-specific character of dominance and suggest that it is primarily dynamic rather than a matter of specific chemical substances.

The holdfasts are also of considerable interest because of their extremely rapid activation and development after the bud stage. The outgrowth of holdfasts within an hour after removal of more proximal regions represents an unusually rapid reaction and the advance of the tip in later stages, even after isolation from the stem may be as rapid as, or even more rapid than the early elonga-

tion. The question whether elongation of the holdfast is due to actual growth, or, as Torrey ('04, '07) believes, to the amœboid activity of the tip, or as to the relative importance of these two factors, cannot be finally answered at present, but it seems evident that the earlier elongation of the holdfast, which may take place free in the water without contact of the tip cannot be due to the amœboid activity of the tip. Migration of cells from the stem into the holdfast is possible, but there is no evidence that it occurs. To all appearances this elongation is due to real growth. As regards the later advance of the tip after isolation and while it is undergoing reduction and atrophy of the more proximal regions is occurring, only further investigation can determine to what extent growth of the tip at the expense of other parts and to what extent amœboid activity is concerned. But whatever the processes underlying particular aspects of its behavior, the holdfast originates as a local region of some sort of physiological activity which decreases peripherally from a center. This activity leads to outgrowth and elongation and the central, most active region necessarily becomes the tip of the outgrowth and the radial gradient becomes an axial gradient. All the behavior of the holdfast is an expression of that fact.

SUMMARY.

1. The holdfasts of *Corymorpha* are tertiary axial gradients developing as buds from the secondary gradient of the basal region.
2. Their premature appearance and outgrowth in embryonic development under inhibiting conditions, as compared with their much later appearance and slower growth in development under normal conditions, suggest that a certain degree of physiological isolation from apical dominance is necessary for their development.
3. As the holdfast elongates, the cœnosarc of its proximal region atrophies so that the distal portion becomes completely isolated from the parent stem, but advance of the tip continues until exhaustion occurs, the distal cœnosarc remaining in good condition while atrophy continues at the proximal end. The delicate perisarc tube secreted as the tip advances is the only connection with the stem in later stages.

4. Holdfast elongation is extremely rapid, sometimes 1 mm. in 15-20 minutes. After a few hours the rate of elongation decreases and exhaustion occurs after 2-3 days. The maximum lengths observed are 2-3 cm.

5. The holdfast tip in contact shows some amœboid activity and while the earlier stages of elongation are apparently due largely or wholly to growth and may occur with the tip free in the water, the continued advance over the substratum after isolation from the stem and while undergoing decrease in size may be due in part to the amœboid activity of the tip.

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